Expanded View Figures

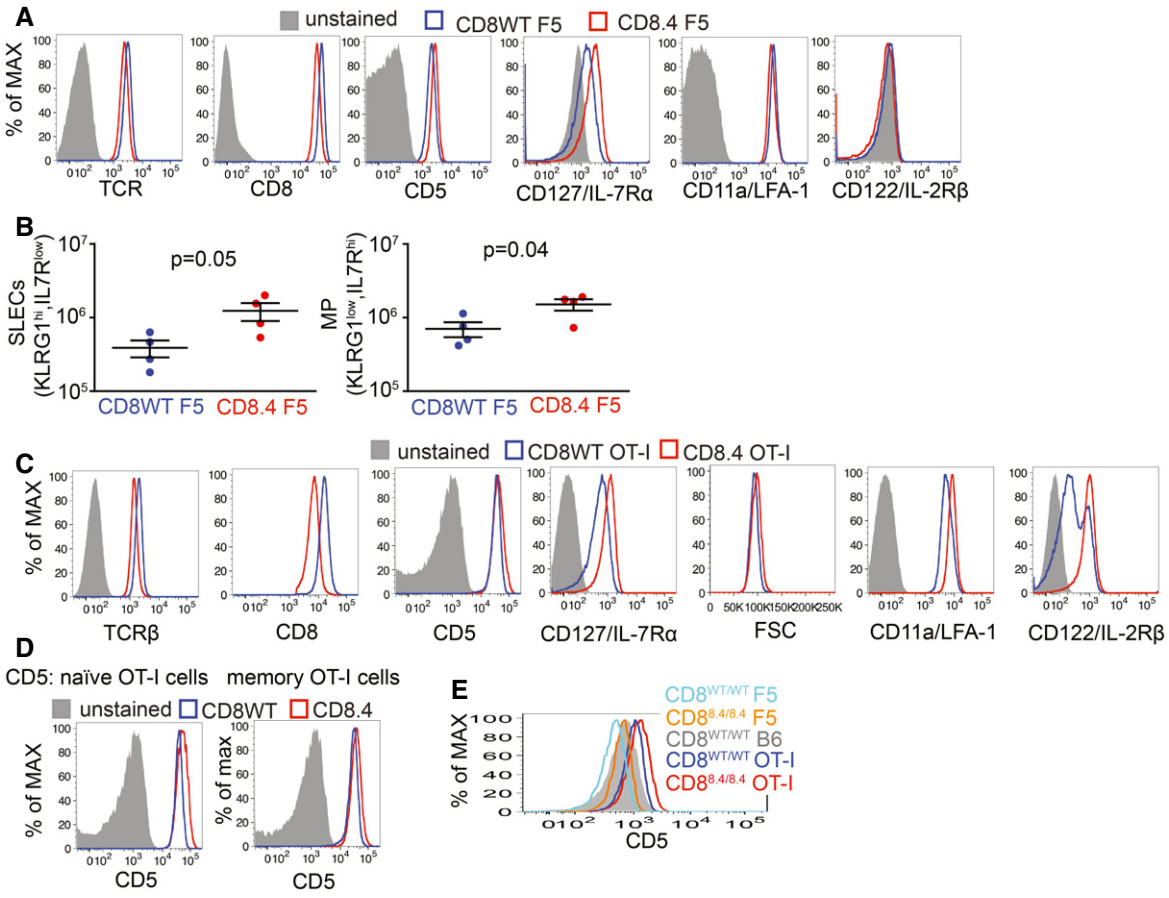

Figure EV1. Analysis of CD8WT and CD8.4 monoclonal T cells, related to Fig 1.

A Expression of indicated surface markers on CD8WT F5 and CD8.4 F5 LN T cell was analyzed by flow cytometry. A representative experiment out of four in total.

B CD8WT F5 and CD8.4 F5 T cells primed by Lm-NP68 (Fig 1D) were examined by flow cytometry. Absolute numbers of KLRG1$^+$ IL-7R$^-$ short-lived effector cells and KLRG1$^-$ IL-7R$^+$ memory precursors were determined. Mean ± SEM. n = 4 mice per group from two independent experiments. Statistical significance was determined by two-tailed t-test. The data do not seem to be strongly deviated from the normal distribution and seem to have equal variance. However, because of the low n, we could not test the normality/equal variance rigorously.

C Expression of indicated markers on CD8WT OT-I and CD8.4 OT-I LN T cell and their size estimated by FSC signal was analyzed by flow cytometry. A representative experiment out of 10 in total.

D Expression of CD5 on CD44$^-$ (naïve) and CD44$^+$ (memory subsets) of CD8WT OT-I and CD8.4 OT-I LN T cells was analyzed by flow cytometry. A representative experiment out of three in total.

E Expression of CD5 on T cells isolated from LN of indicated mouse strains by flow cytometry. A representative experiment out of two in total.

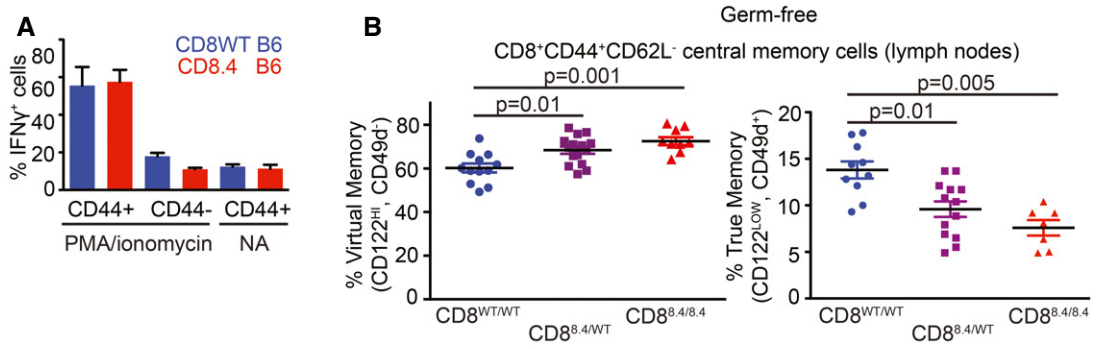

Figure EV2. Analysis of CD8WT and CD8.4 polyclonal T cells, related to Fig 2.

A LN cells isolated from polyclonal CD8WT and CD8.4 mice were stimulated with 10 ng/ml PMA and 1.5 μM ionomycin or left untreated for 5 h in the presence of BD Golgi Stop and the percentage of IFNγ-producing cells was determined by flow cytometry. The cells were gated as $CD8^+$ and further divided into $CD44^+$ and $CD44^-$ populations. Mean + SEM. n = 4 independent experiments.

B Quantification of the frequency of $CD122^{HI}$ $CD49d^-$ VM and $CD122^{LOW}$ $CD49^+$ true memory cells among $CD8^+$ $CD44^+$ $CD62L^+$ CM T cells isolated from LN is shown. Mean, n = 9–15 mice per group from four independent experiments. Statistical significance was determined using two-tailed Mann–Whitney test.

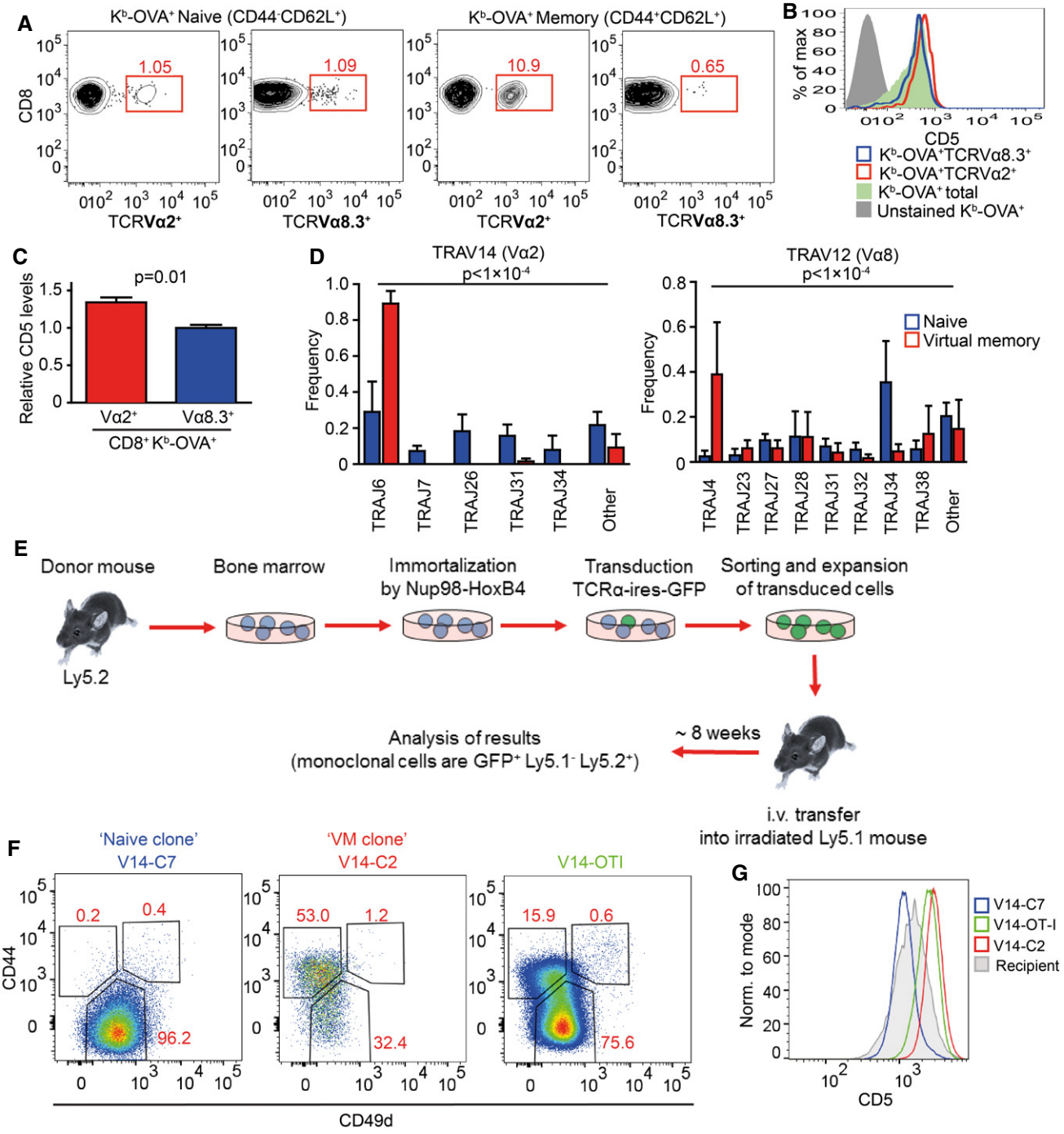

Figure EV3. Differences in TCR repertoires between naïve and VM T cells, related to Fig 3.

A Alternative gating strategy for the same samples as shown in Fig 3B–D (T cells from Vβ5 mice). A representative experiment out of eight in total.

B, C The expression of CD5 on $CD8^+$ K^b-OVA-4mer$^+$ $CD8^+$ $V\alpha2^+$ or $V\alpha8.3^+$ cells or total $CD8^+$ K^b-OVA-4mer$^+$ isolated from LN and the spleen was determined by flow cytometry. Relative MFI CD5 levels were quantified (CD5 levels on $CD8^+$ K^b-OVA-4mer$^+$ $V\alpha8.3^+$ were arbitrarily set as 1). Mean + SD, n = 3 independent experiments. Statistical significance was determined using two-tailed one-value t-test.

D TCRα sequences from Fig 3F were analyzed for TRAJ usage. Means + SEM. n = 4 independent experiments. Statistical significance was determined by chi-square test.

E Schematic representation of the generation and analysis of retrogenic monoclonal T-cell subsets.

F Frequency of VM T cells among monoclonal populations expressing TCR clones V14-C2, V14-C7, and OT-I generated as in Fig 3G.

G CD5 levels on naïve monoclonal T cells expressing V14-C2, V14-C7, and OT-I. Representative mice out of 9–14 in total from two to four independent experiments.

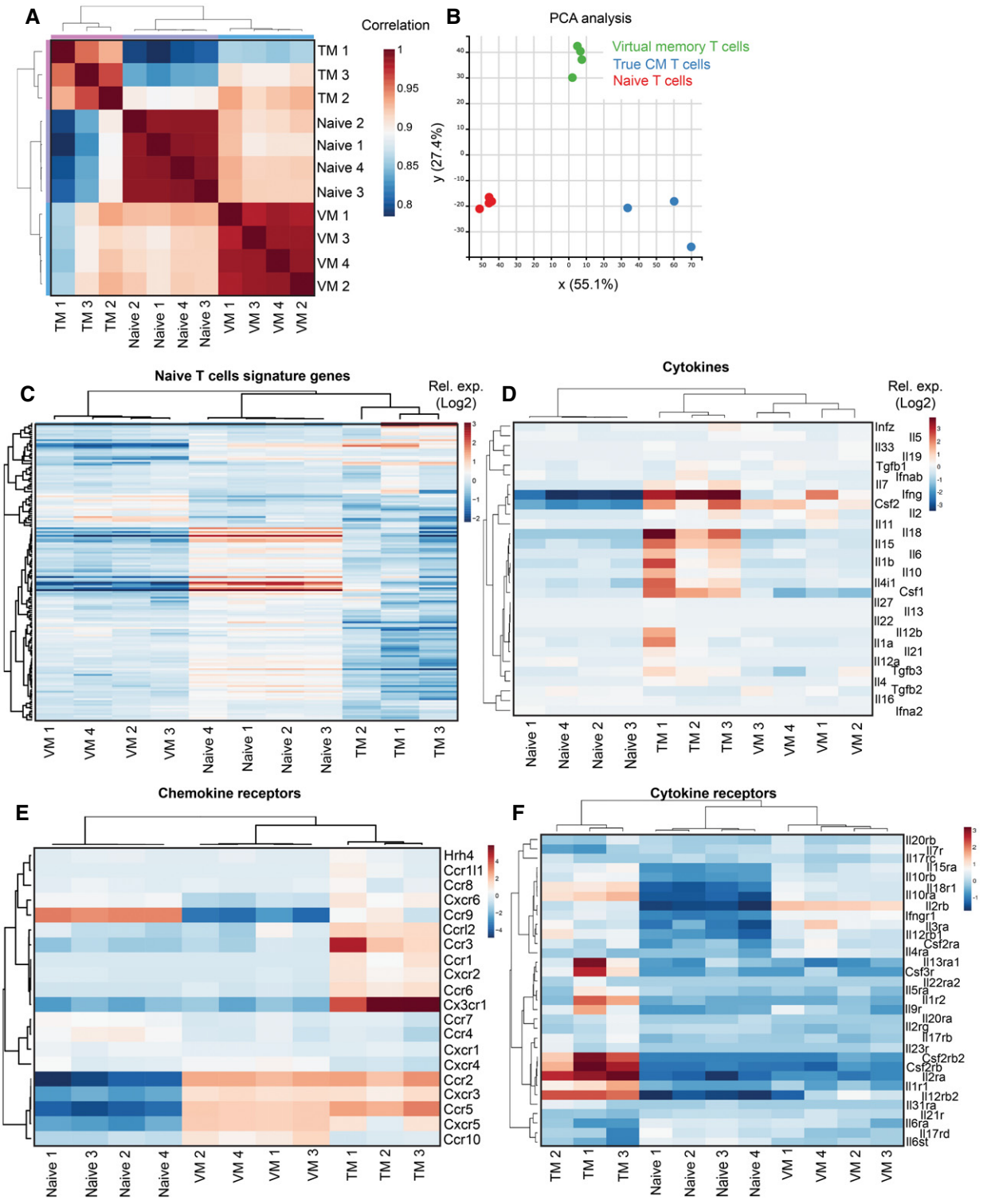

Figure EV4. Gene expression analysis of naïve, VM, and TM T cells, related to Fig 4.

Transcriptomes of naive (n = 4), VM (n = 4), and TM (n = 3) CD8$^+$ T cells were analyzed by deep RNA sequencing.

A Correlation of the gene expression between individual samples.

B PCA analysis between the samples.

C–F Enrichment of CD8$^+$ naïve signature genes (as revealed by previous studies) (C), cytokine encoding genes (D), chemokine receptor encoding genes (E), and cytokine receptor encoding genes (F) in naïve, VM, and true CM T cells.

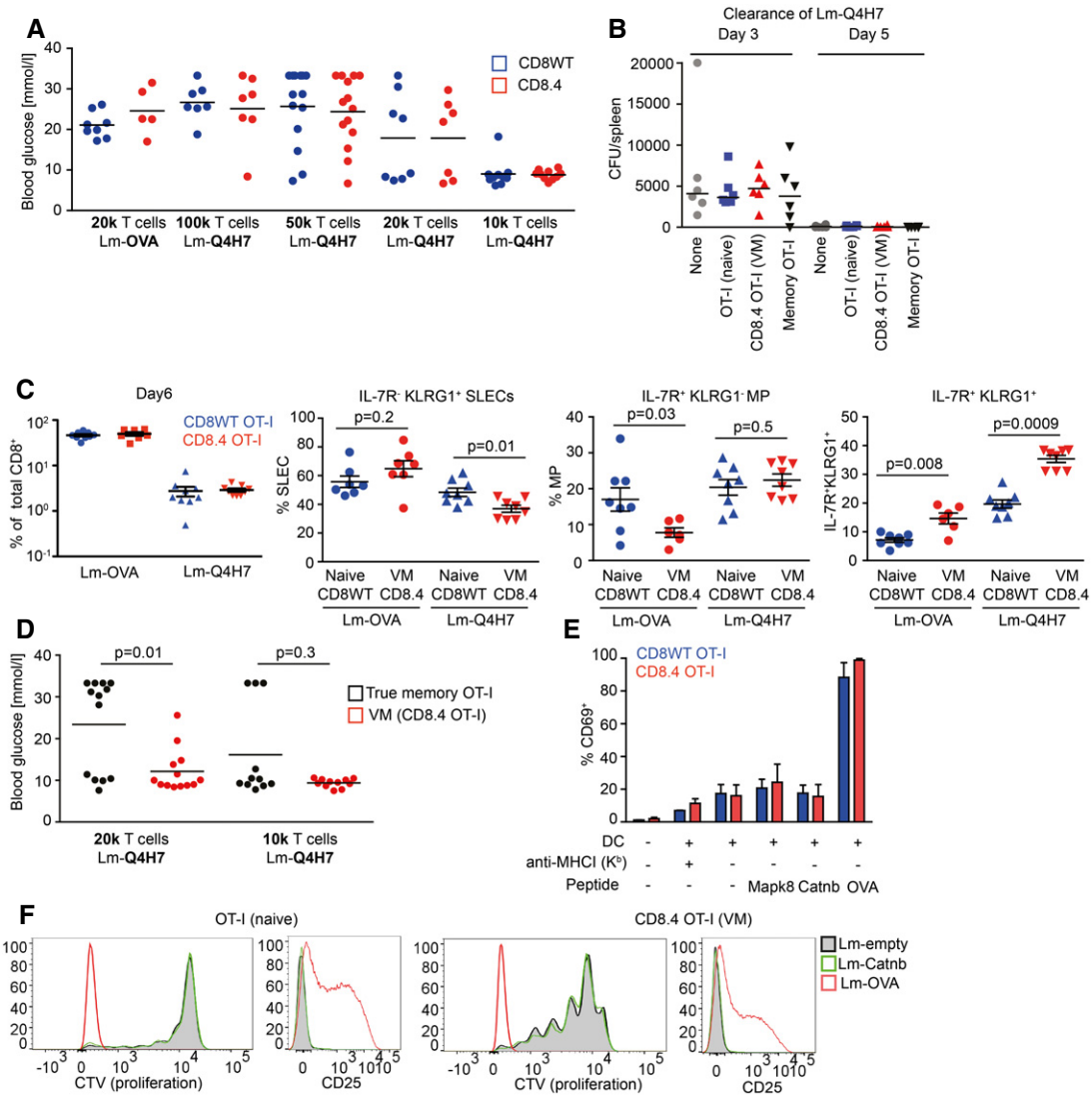

Figure EV5. Functional characterization of naïve, VM, and TM T cells, related to Fig 5.

A Indicated number of CD8WT OT-I or CD8.4 OT-I T cells were adoptively transferred into RIP-OVA hosts, which were infected with Lm-OVA or -Q4H7 1 day later. Blood glucose was measured 7 days after the infection. n = 5–11 mice per group in 3–6 independent experiments. These are the same experiments as in Fig 5A. Mean is indicated.

B FACS-sorted 1 × 10^4 naïve CD8WT OT-I, CD8.4 OT-I (VM), sorted true memory OT-I T cells or no T cells were adoptively transferred into Ly5.1 C57Bl/6 mice followed by infection with 5,000 CFU Lm-Q4H7. Total Lm-Q4H7 CFU counts in the spleen were quantified on day 3 and day 5 post-infection. n = 6 mice in two independent experiments.

C Quantification of the experiment shown in Fig 5E and F. Percentage of donor OT-I and CD8.4 OT-I T cells among all $CD8^+$ T cells is shown. Percentage of short-lived effector cells ($IL\text{-}7R^-$ $KLRG1^+$), memory precursors ($IL\text{-}7R^+$ $KLRG1^-$), and double-positive cells $IL\text{-}7R^+$ $KLRG1^+$ among the donor cells are shown. Statistical significance was calculated using Mann–Whitney test. n = 7 (CD8.4 OT-I + Lm-OVA) or 9 (the other three experimental conditions) in four independent experiments.

D Indicated number of CD8.4 OT-I or true memory OT-I T cells were adoptively transferred into RIP-OVA hosts, which were infected with Lm-Q4H7 1 day later. The glucose in the blood was measured on day 7 post-infection. Statistical significance was calculated using Mann–Whitney test n = 11 (1 × 10^4 transferred cells) or 13 (2 × 10^4 transferred cells) mice per group in four independent experiments. These are the same experiments as in Fig 5H.

E OT-I or CD8.4 OT-I was cocultured with bone marrow dendritic cells that were pre-loaded or not with 10 μM putative endogenous positive selecting peptides for OT-I T cells (Mapk8, AGYSFEKL; Cantb, RTYTYEKL) or with 0.1 μM OVA peptide. Blocking of the MHCI by adding anti-H2-K^b antibody (clone Y3, 10 μg/ml) was used as a negative control. Percentage of $CD69^+$ T cells in the overnight stimulation was quantified by flow cytometry. Mean + SEM. n = 2 (MHC-I blocking) or 3 (all the other experimental conditions) independent experiments.

F CellTrace violet-loaded OT-I or CD8.4 OT-I T cells were adoptively transferred into Ly5.1 C57Bl/6 donors followed by infection with Lm-OVA (1 × 10^4 transferred cells), Lm-Catnb, or empty Lm (both 1 × 10^5 transferred cells) 1 day later. Expression of activation marker CD25 and dilution of the proliferation dye in donor cells was examined on day 5 post-infection. Representative mice out of seven in total from three independent experiments are shown. The cells from the Lm-OVA infected mice are the same as used for the analysis in Fig 5G.